

EXPERIMENTAL INFECTION OF *Macaca rhesus*
WITH L-FORMS OF HEMOLYTIC STREPTOCOCCUS

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UDC 616.981.214-092.9-078:576.851.214.095.5

Blood cultures 1-2, 7-8, and 20 days after infection of macaques (*Macaca rhesus*) with L-forms of hemolytic streptococcus were negative for these forms. Cultures of homogenates of the liver, kidneys, spleen, and heart at the same times after infection gave infrequent growth of L-colonies. In monkeys infected intramuscularly, L-colonies were formed more frequently than in animals infected intravenously. The isolated L-colonies were of low viability and survived only 2-3 subcultures. They were found on the 7th-8th day in homogenates of the liver, on the 7th-8th and 20th days in homogenates of the spleen, and at all times of investigation in homogenates of the kidneys.

Previous investigations showed that experimental tonsillitis complicated by infectious myocarditis can be reproduced in macaques (*Macaca rhesus*) infected with L-forms of group A hemolytic streptococcus [2-4]. At the same time, the frequency of isolation of L-forms from the blood of the infected animals was extremely low. No conclusions could be drawn from the results regarding the possible long latency of the organism in the L-forms.

The investigation described below was carried out to investigate the duration of survival of L-form of streptococcus in the body.

EXPERIMENTAL METHOD

The experimental animals were 28 adult monkeys weighing 3-5 kg which were infected with cultures of stable L-forms of group A streptococcus (strain L-49 obtained from Dr. Schmitt-Elimska from the International Children's Center, Paris, and L-406 isolated by G. Ya. Kagan and V. S. Mikhailova from the blood of a patient with rheumatic carditis in Moscow). The same strains heated for 1 h at 60°C and the cultivation medium were used as the control.

Depending on the method of injection the monkeys were divided into three groups: Group 1) intravenous injection of a culture of L-forms: a) unheated cultures: 5 monkeys received L-49 and 4 received L-406; b) heated cultures: 2 monkeys received L-49 and 2 received L-406; c) 1 monkey received the culture medium. Group 2) intramuscular injection of the same strains: a) unheated cultures: 4 monkeys received L-49 and 4 received L-406; b) heated cultures: 4 monkeys received both strains; c) 1 monkey received the culture medium. Group 3) 1 uninfected control monkey.

The method of preparation of suspensions of the L-forms for infection was described previously [4, 1], and the infecting dose was 1 ml (10^{10})/kg body weight. Intravenous injections were given daily for 4 days, intramuscular injections were given twice at an interval of 4-5 days. Blood cultures were taken after each injection and thereafter once a week throughout the period of observation. The monkeys were autopsied on

N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. D. Timakov.) Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 74, No. 7, pp. 79-81, July, 1972. Original article submitted December 31, 1971.

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TABLE 1. Results of Isolation of L-forms and Granular Unidentified Structures from Blood and Organs of Monkeys Infected with L-Forms of Hemolytic Streptococcus (strains L-406 and L-49)

Method of injection	Material injected	Number of monkeys	Form iso-lated	Number of animals with positive results of culture after infection													
				First-second day				7th-8th day				20th day					
				blood	liver	Kid-neys	spleen	heart	blood	liver	Kid-neys	spleen	heart	blood	liver	Kid-neys	spleen
Intravenously	Strains L-406 and L-49	9	L 3	0/9 0/9	0/3 0/2	0/2 1/2	1/2 1/2	0/2 1/2	0/7 0/7	0/3 2/2	0/2 1/2	1/2 2/2	6/2 1/2	0/5 0/5	1/2 1/2	0/2 1/2	0/2 1/2
Intramuscularly		8	L 3	0/8 0/8	0/2 1/2	1/2 1/2	0/2 2/2	0/2 1/2	0/6 0/6	1/3 1/2	2/2 0/2	1/2 1/2	0/2 0/2	0/4 0/4	0/1 0/2	1/1 0/2	0/1 0/2
Intravenously	Heated cultures (the same strains)	4	L 3	0/4 0/4	0/2 0/2	0/2 2/2	0/2 2/2	0/2 1/2						0/2 0/2	0/2 0/2	0/2 1/2	0/2 0/2
Intramuscularly		4	L 3	0/4 0/4	0/2 0/2	0/2 0/2	0/2 2/2	0/2 1/2						0/2 0/2	0/1 0/1	0/1 0/1	0/1 0/1
Intramuscularly	Culture medium	1	L 3	0/1 0/1					0/1 0/1	0/1 0/1	0/1 0/1	0/1 0/1	0/1 0/1				
		1	L 3	0/1 0/1	0/1 0/1	0/1 0/1	0/1 0/1										

Notes: Number of monkeys with positive cultures shown as numerator, total number investigated as denominator. L) L-colonies, G) colonies of granular forms.

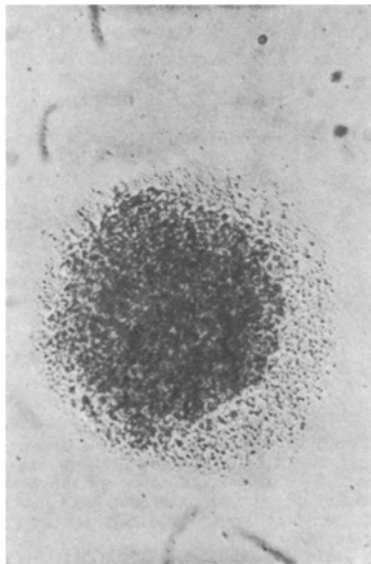


Fig. 1. L-colony, 302 \times .

the 1st-2nd, 7th-8th, and 20th days after the last infecting dose and culture taken of homogenates of the organs. The method of obtaining the cultures was described previously [2, 4, 5].

EXPERIMENTAL RESULTS

The results of cultures of blood and organ homogenates of the experimental and control animals (Table 1) are as follows: L-forms were extremely rarely isolated from organ homogenates of the infected monkeys and never from blood cultures. The typical L-colonies isolated (Fig. 1) either did not survive subculture or survived only 2-3 subcultures on media with an inducing agent (penicillin 1000 units/ml) and they did not revert in the course of 10 successive subcultures on media without the agent inducing the L-form. It was therefore impossible to determine the species of the colonies grown.

As in the previous experiments growth was observed in the form of granules, which developed over a period of 2 or 3 subcultures without reversion to the bacterial forms and without the formation of typical L-colonies. The bacterial forms and without the formation of typical L-colonies. The bacterial nature of these granules and the species to which they belonged could not be determined by bacteriological methods.

Analysis of Table 1 shows that after intramuscular injection of L-forms into the monkeys L-colonies were found rather more frequently than by the intravenous route. They were isolated from the kidneys at all times of investigation, they were found on the 7th-8th day in the liver, and on the 7th-8th and 20th days in the spleen. Colonies of granular forms were isolated at nearly all times in large numbers after intravenous injection. L-colonies were found on the 1st-2nd and 7th-8th day in the spleen and on the 20th day in the liver.

The predominance of colonies of granules after intravenous injection of the L-forms may be the result of their disintegration in the blood stream and subsequent deposition in the organs.

The experiments thus showed that L-forms and granular forms are found in all organs (liver, kidneys, spleen, heart) from the 1st to the 20th days after infection.

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